High dietary fat intake induces a microbiota signature that promotes food allergy

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Background

Diet-induced obesity and food allergies increase in tandem, but a potential cause-and-effect relationship between these diseases of affluence remains to be tested.

Objective

We sought to test the role of high dietary fat intake, diet-induced obesity, and associated changes in gut microbial community structure on food allergy pathogenesis.

Methods

Mice were fed a high-fat diet (HFD) for 12 weeks before food allergen sensitization on an atopic dermatitis–like skin lesion, followed by intragastric allergen challenge to induce experimental food allergy. Germ-free animals were colonized with a signature HFD or lean microbiota for 8 weeks before induction of food allergy. Food-induced allergic responses were quantified by using a clinical allergy score, serum IgE levels, serum mouse mast cell protease 1 concentrations, and type 2 cytokine responses. Accumulation of intestinal mast cells was examined by using flow cytometry and chloroacetate esterase tissue staining. Changes in the gut microbial community structure were assessed by using high-throughput 16S ribosomal DNA gene sequencing.

Results

HFD-induced obesity potentiates food-induced allergic responses associated with dysregulated intestinal effector mast cell responses, increased intestinal permeability, and gut dysbiosis. An HFD-associated microbiome was transmissible to germ-free mice, with the gut microbial community structure of recipients segregating according to the microbiota input source. Independent of an obese state, an HFD-associated gut microbiome was sufficient to confer enhanced susceptibility to food allergy.

Conclusion

These findings identify HFD-induced microbial alterations as risk factors for experimental food allergy and uncouple a pathogenic role of an HFD-associated microbiome from obesity. Postdieting microbiome alterations caused by overindulgence of dietary fat might increase susceptibility to food allergy.

Key words: Food allergy; diet-induced obesity; high-fat diet; germ-free; microbiota; dysbiosis; basophils; mast cells; IgE

Abbreviations used: AD, Atopic dermatitis; FITC, Fluorescein isothiocyanate; GF, Germ-free; H&E, Hematoxylin and eosin; HFD, High-fat diet; MCp, Mast cell progenitor; MMCP-1, Mouse mast cell protease 1; NMDS, Nonmetric multidimensional scaling; OTU, Operational taxonomic unit; OVA, Ovalbumin; SCD, Standard chow diet; SLN, Skin-draining lymph node; WAT, ~~W~~white adipose tissue

The past few decades have witnessed a continuous increase in the incidence and severity of food allergies, suggesting that modern lifestyle choices can promote disease in genetically susceptible subjects.[1] Various hypotheses have been formulated to explain this phenomenon, with the hygiene hypothesis and diet hypotheses attracting the most attention.[2,3] Reduced exposure to environmental microorganisms and changes in our dietary habits associated with a Western lifestyle have been discussed as potential drivers of this epidemiologic trend.[4-8] Although a modern diet might ensure protection from micronutrient and macronutrient deficiencies, overindulgence in refined sugars and saturated fat can contribute to the ever-growing increase in immune-mediated disorders, including allergies.[9] Despite epidemiologic data identifying a link between obesity and allergic inflammation,[10-13] how diet and obesity can influence food allergy remains to be tested.

A potential link between diet and food allergy is the intestinal microbiota. Consumption of a Western diet has a profound effect on our gut ecosystems.[14-20] Dietary intake of high-fat, high-sugar, low-fiber foods along with therapeutic use of antibiotics perturbs naturally selected host-microbe interactions.[21-25] Given that the microbiota is involved in regulation of numerous aspects of normal host physiology, changes in this intimate host-microbe interaction might have long-term consequences for the regulation of immunity.[26-31] Many studies have clearly defined a relationship between alterations in the gut microbial community structure and allergic disorders, such as food allergy.[32,33] Independently, recent advances in microbiome biology have identified that dietary changes promote microbial alterations associated with obesity and metabolic derangements.[34-37] Importantly, changes in core microbial community structure associated with obesity or food allergy have been demonstrated to confer a disease phenotype, highlighting the pathogenic role of a dysbiotic state in these settings.[21,27,32,33] However, whether high-fat diet (HFD)–induced changes in the gut microbiome influence food allergy susceptibility remains unknown. Furthermore, whether such changes in the gut microbial community structure regulate allergic disease through alteration of host metabolism or through direct effects on the host's immune system remains unclear. Thus the focus of the current study was to clearly define how diet and associated gut microbial alterations influence food allergy.

To do so, we used a previously established model of intestinal food allergy and manipulated dietary composition using a well-defined model of HFD feeding.[38-42] Using high-throughput 16S rDNA sequencing, we assessed gut microbial community structure in response to HFD feeding and in the setting of experimental food allergy. Finally, we examined whether HFD-induced changes in the gut commensal community structure alter susceptibility to food allergy. Taking these unique approaches, we identified that HFD-induced gut microbial alterations promote food allergy independently of obesity.

Methods

Mice

Age-, sex-, and strain-matched control mice were used in all experiments. Wild-type specific and opportunistic pathogen-free C57BL/6J mice were provided by Janvier Labs (Paris, France) and housed under specific and opportunistic pathogen-free conditions at the central animal facility of the Medical School of the University of Bern. For diet-induced obesity, mice were fed an HFD (45 kcal% fat, D12451i; Research Diets, New Brunswick, NJ) for up to 12 weeks before induction of experimental food allergy. Control groups were fed a standard autoclavable rodent chow (standard chow diet [SCD]; 4.5 kcal% fat; Provimi Kliba SA, Penthalaz, Switzerland) or a matched diet (10 kcal% fat, D12450H; Research Diets). All diets were gamma-irradiated (10-20 kGy). For microbiota transfer studies, 4-week-old germ-free (GF) mice were cohoused with mice fed an HFD or SCD diet for 8 weeks before induction of food allergy. All animal experiments were performed in compliance with Swiss Federal regulations.

Experimental food allergy

Mice were treated daily with 2 nmol of MC903 (calcipotriol; Tocris Bioscience, Bristol, United Kingdom) in 20 μL of 100% EtOH on both ears (apical surface) in the presence of 100 μg of ovalbumin (OVA) for 14 consecutive days to induce experimental food allergy. As a vehicle control, the same volume of EtOH and OVA was applied. All mice were starved for 12 hours before intragastric antigen challenge with 50 mg of OVA on days 14 and 17.5 and killed 12 hours after the final oral antigen challenge. Starting 20 minutes after oral gavage, mice were individually observed for a period of 30 minutes, and a clinical allergy score was assessed (see Table E1 in this article's Online Repository

at www.jacionline.org). Investigators were blind to treatment group conditions when scoring for allergy symptoms. Ear thickness was measured after treatment by measuring the entire width of the ear at the widest point in microns from the top of one stratum corneum to the top of the stratum corneum on the opposite side of the ear.

Isolation of cells

At necropsy, single-cell suspensions of skin-draining lymph nodes (SLNs) were prepared by passing through 70-μm nylon mesh filters. Small intestinal lamina propria cells were isolated by thoroughly washing the small intestines in PBS and incubating for 10 minutes at 37°C with 5 mL of epithelial stripping buffer containing 5 mmol/L EDTA, 2 mmol/L dithiothreitol, and 5% FCS in PBS solution with shaking. Samples were thoroughly vortexed, and remaining tissues were digested in 5 mL of serum-free medium containing 1 mg/mL collagenase type IV (Sigma, St Louis, Mo) and 10 U/mL DNase I (Roche, Mannheim, Germany) for 30 minutes at 37°C on an orbital shaker. Digested tissues were passed through 70-μm nylon mesh filters, and single-cell suspensions were stained with biotin anti-mouse epithelial cell adhesion molecule (G8.8; BioLegend, San Diego, Calif) for targeted removal of epithelial cells with Streptavidin RapidSpheres and separation with an EasySep magnet (STEMCELL Technologies, Vancouver, British Columbia, Canada).

Flow cytometry

Single-cell suspensions of indicated tissues were incubated with Aqua Live/Dead Fixable Dye (Invitrogen, Carlsbad, Calif) for dead cell exclusion and stained with anti-mouse fluorochrome-conjugated mAbs against CD3ε (145-2C11), CD4 (GK1.5), CD5, CD8, CD19 (1D3), NK1.1 (PK136), CD19 (6D5), IgE, CD45 (30-F11), CD49b (DX5), c-Kit (2B8), and FcεRI (Mar-1). Intestinal mast cells were identified as live Lin^- ($CD3^-CD5^-CD19^-NK1.1^-$), $CD45^+$, $c\text{-}Kit^+$, and IgE^+ cells. Intestinal eosinophils were identified as live Lin^-, $CD45^+$, side scatter–high, and $Siglec\text{-}F^+$ cells, and skin basophils were identified as live Lin^-, $CD45^+$, and $c\text{-}Kit^-$ cells coexpressing CD49b and IgE. All samples were acquired on an LSRII (BD Biosciences, San Diego, Calif) and analyzed with FlowJo software (TreeStar, Ashland, Ore).

Cell stimulation

Single-cell suspensions from SLNs were cultured with 100 μg of pyrogen-free OVA (Hyglos, Bernried am Starnberger See, Germany) for 96 hours in complete media (RPMI 1640 containing 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mg/mL L-glutamine, and 25 mmol/L HEPES). Cell-free supernatants were collected for determination of type 2 cytokine protein levels by using a standard sandwich ELISA.

ELISA, histology, RNA isolation, and quantitative real-time RT-PCR

Measurement of levels of mouse mast cell protease 1 (MMCP-1) in serum was performed according to a commercially available DuoSet ELISA kit (eBioscience, San Diego, Calif). Total and antigen-specific serum IgE responses were measured, as described previously.[40]

For histologic analysis, tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E). H&E-stained epididymal adipose tissue sections were digitalized by using the Pannoramic 250 Flash III (3DHISTECH, Budapest, Hungary) whole-slide scanner equipped with a ×20 objective. H&E-stained epididymal adipose tissues were analyzed by determining the area of each adipocyte in the field (×20 magnification). Three fields were randomly selected for analysis from each tissue section from each mouse. Adipocytes were categorized by size, and the average number of adipocytes per size was determined. Data were expressed as the frequency of adipocytes in each size category compared with the total number of adipocytes counted.

Intestinal mast cells were stained with Naphtol AS-D Chloroacetate–specific esterase (Sigma Aldrich), according to the manufacturer's instructions. Tissue samples were homogenized in TRIzol with a TissueLyser (Qiagen), and RNA was isolated by using phenol chloroform extraction and isopropanol precipitation. cDNA was generated with the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, Calif). RT-PCR was performed by using commercially available QuantiTect primer assays (Qiagen). Data were analyzed by using the ΔΔ cycle threshold method whereby β-actin served as an endogenous control. All samples were run with RT SYBR Green on an Applied Biosystems 7900HT Fast Real-time PCR system.

Intestinal permeability

Intestinal permeability was assessed by using fluorescein isothiocyanate (FITC)–Dextran accumulation in serum. Mice were fasted for 4 hours before oral gavage with 60 mg/100 g mouse 4-kDa FITC-Dextran (Sigma-Aldrich). Four hours after oral gavage, blood was collected by means of cardiac puncture, stored on ice in the dark, and centrifuged at 10,000*g* for 10 minutes. Serum was diluted 1:10 in PBS, and the concentration of FITC-Dextran was determined by using fluorescence spectroscopy (excitation at 485 nm and emission at 535 nm). For calculation of a linear standard curve, FITC-Dextran was serially diluted in sera of naive mice.[43]

Bacterial DNA isolation and 16S rDNA gene sequence analysis

Stool samples from individual mice were collected fresh into sterile 2-mL microcentrifuge tubes at the indicated time points, snap-frozen, and stored at −80°C until DNA isolation. Fecal genomic DNA was prepared by using the

QIAamp Fast DNA Stool Mini Kit (Qiagen), according to the manufacturer's instructions, with minor modifications. Additional homogenization (glass beads at 30 Hz for 3 minutes) and lysis were performed (20 mg/mL Lysozyme [Sigma-Aldrich], 20 mmol/L Tris HCl [pH 8.0], 2 mmol/L EDTA, and 1.2% triton autoclaved) to improve the quality and quantity of DNA from both gram-negative and gram-positive bacteria. Fecal DNA was then extracted according to the Kit protocol (QIAmp Fast DNA Stool Mini Kit, Qiagen). The concentration and purity of the isolated DNA were evaluated by using NanoDrop and Bioanalyzer (Thermo Scientific, Waltham, Mass). PCR amplification of 16S rDNA genes with primers specific for the V4 domain of bacterial rDNA (341F_ill: CCTACGGGNGGCWGCAG and 802R_ill: GACTACHVGGGTATCTAATCC), purification of PCR products, library preparation, and sequencing on the Illumina MiSeq system were performed by Microsynth AG (Balgach, Switzerland).

~~Raw reads were quality filtered with FastX Tools (Q 33, -p 75), merged with PEAR (-m 360 -n 100 -j 4 -q 26 -v 50 -b 33; Zhang et al, 2014~~)Raw reads were quality-filtered with FastX-Tookit (a minimum quality score of 33 kept across 75% of the bases), merged with PEAR (with a maximum possible length of merged reads of 360, minimum possible length of merged reads of 100, score quality threshold for trimming of 26, minimum overlap size of 50, and a base PHRED quality score of 33 and filtered again with VSEARCH fastq _filter. Reads were dereplicated (-minuniquesize 2), clustered at 0.97 identity, and chimera filtered with VSEARCH.[44] Filtered reads were then mapped back to the final clusters with VSEARCH —usearch_global. The resulting operational taxonomic unit (OTU) table was normalized by using total DNA extraction yields, and samples with low output values were excluded from the analysis. Representative sequences (centroids) from each cluster were classified with SINA against the SILVA_132_NR99 database,[45] and composition was explored by using PHINCH.[46] Differences between treatments were detected with the Welch 2-sided test, and significantly different taxa were considered when the *P* value was less than .05 (corrected with the Storey false discovery rate) and effect sizes were greater than 1 for differences between proportions and greater than 2 for ratios between proportions. Statistical differences were calculated with STAMP.[47] Normalized OTU tables were used to calculate rarefactions, richness (total OTU number), and the Shannon index. A Hellinger-corrected Bray-Curtis similarity matrix was used to calculate an nonmetric multidimensional scaling (NMDS) and a cluster analysis, and both ADONIS and ANOSIM were used to test for differences between treatments. All calculations were done in the R package VEGAN.

Statistical analysis

Except for the 16S rDNA gene sequence analyses, all data were analyzed with GraphPad Prism 6 software (GraphPad Software, La Jolla, Calif). Results are shown as means ± SEMs. Statistical significance was determined by using the Student *t* test or 1-way ANOVA with the Tukey or Dunn multiple comparison tests. Results were considered significant at *P* values of .05 or less. The number of experiments performed is indicated at the end of each figure legend.

Results

High dietary fat intake potentiates food-induced allergic responses

The increasing prevalence of obesity and food allergy provokes the hypothesis that these processes might be related. Although obesity has been associated with increased IgE responses and allergic symptoms, whether it predisposes to an increased food allergy risk remains to be tested.[48-51] To investigate whether HFD feeding confers an increased risk of food allergy, wild-type C57BL/6J mice were fed an HFD (45 kcal% fat, D12451i; Research Diets), a matching control diet (10 kcal% fat, D12450H; Research Diets), or an SCD (4.5 kcal% fat; Provimim Kliba SA) for 12 weeks before induction of experimental food allergy (Fig 1, *A*). After establishment of obesity in the HFD group (Fig 1, *B-D*, and see Fig E1 in this article's Online Repository at www.jacionline.org), mice were sensitized to the model food antigen OVA on atopic dermatitis (AD)–like skin lesions. Then, as previously described,[38,40] mice were challenged intragastrically with OVA to induce food allergy. This resulted in phenotypic features of food allergy, as characterized by an increased clinical food allergy score (Fig 1, *E*), total and antigen-specific IgE responses (Fig 1, *F* and *G*), and serum MMCP-1 levels (Fig 1, *H*). In contrast to mice with food allergy fed an SCD, mice with food allergy fed an HFD exhibited an increased food allergy score (Fig 1, *E*) and increased serum MMCP-1 levels (Fig 1, *H*), whereas no significant changes in total and allergen-specific serum IgE levels were observed between treatment groups (Fig 1, *F* and *G*). Importantly, mice fed a matched control diet exhibited similar allergic responses than mice with food allergy fed an SCD (see Fig E2 in this article's Online Repository at www.jacionline.org).

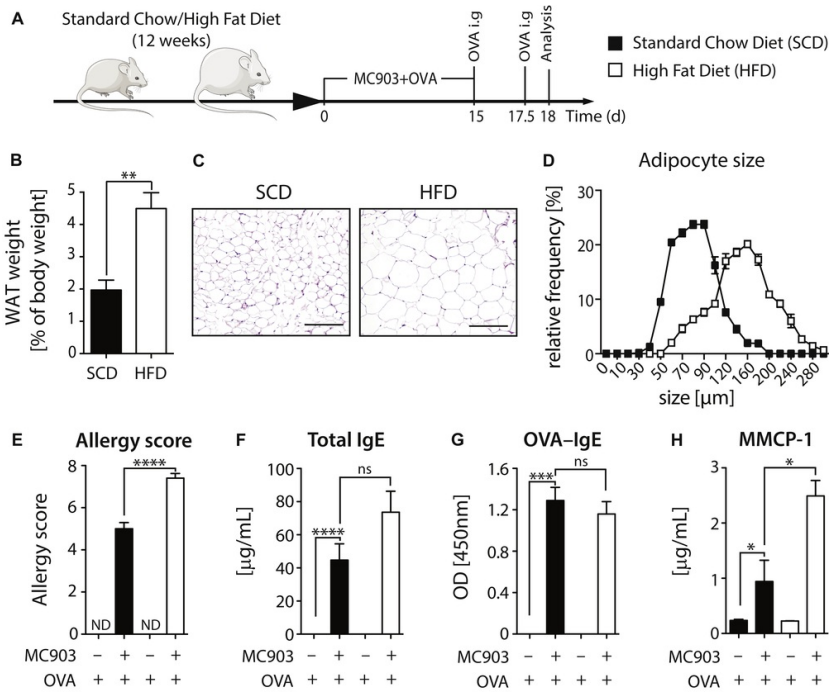

Fig 1 Diet-induced obesity potentiates food-induced allergic responses. **A,** Experimental protocol. *i.g.*, Intragastric. **B,** WAT weight of percentage body weight. **C,** H&E staining of WAT. *Scale bar* = 100 µm. **D,** Distribution of adipocyte size of WAT, as determined by using histomorphometric analyses. **E,** Clinical allergy score of control mice and mice with food allergy (MC903 and OVA) fed an SCD or HFD. **F** and **G,** Total (Fig 1, *F*) and OVA-specific (Fig 1, *G*) serum IgE levels. **H,** Serum MMCP-1 levels. Data are representative of 4 independent experiments with 4 to 6 mice per group. *Solid bars* represent mice fed an SCD, and *open bars* represent HFD-fed mice. *Error bars* indicate means ± SEMs. *ND*, No disease; *ns*, not significant. **P* < .05, ***P* < .01, ****P* < .005, and *****P* < .001.

Together, our data indicate that prolonged feeding of an HFD associated with obesity increased susceptibility to experimental food allergy.

HFD does not alter AD-like skin inflammation and food allergen sensitization

To test whether enhanced food allergy in HFD-fed mice was simply secondary to enhanced skin inflammation, cutaneous food allergen sensitization, or both, we first assessed the development of AD-like skin lesions in response to topical calcipotriol (MC903) and OVA treatment in mice fed an SCD or HFD. Skin inflammation was assessed based on histology (Fig 2, *A*), ear swelling (Fig 2, *B*), and infiltration of total leukocytes (Fig 2, *C*) and basophils (Fig 2, *D*), which were previously shown to promote AD-like skin lesions and cutaneous food allergen sensitization in this model system.[38,40] However, irrespective of disease state and the diet fed, no differences on all parameters assessed were observed (Fig 2, *A-D*).

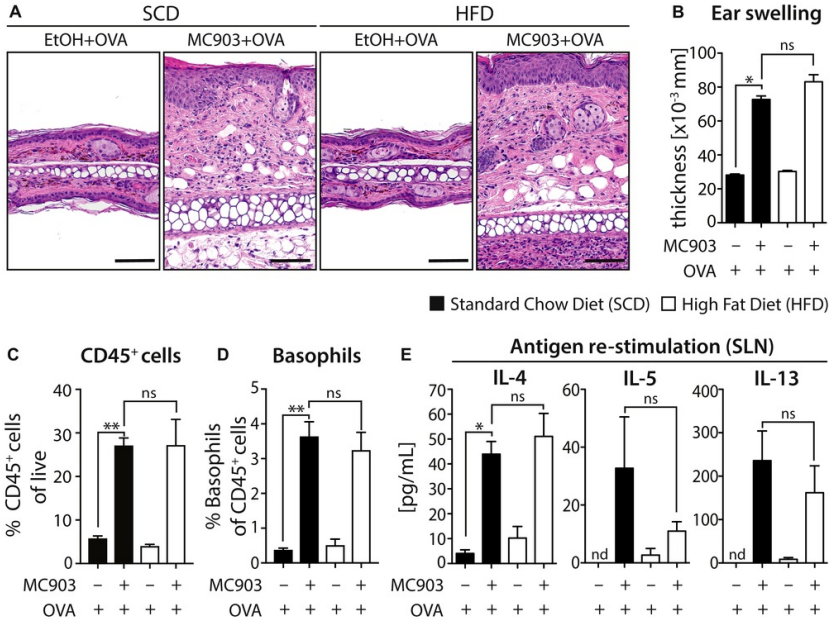

Fig 2 HFD does not alter AD-like skin inflammation and cutaneous food allergen sensitization. **A,** H&E staining of the skin of control mice (EtOH plus OVA) or mice with food-induced allergy (MC903 plus OVA) fed an SCD or HFD. *Scale bar* = 100 μm. **B,** Ear swelling. **C** and **D,** Frequencies of total leukocytes (Fig 2, *C*) and skin basophils (Fig 2, *D*). Basophils were gated as live CD45$^+$, Lin$^-$ (CD3$^-$CD5$^-$CD19$^-$NK1.1$^-$), and c-Kit$^-$ cells coexpressing CD49b and IgE. **E,** Type 2 cytokine production in cell-free supernatants of antigen-restimulated SLN cultures. Data are representative of 3 individual experiments with 4 to 6 mice per group. *Error bars* indicate means ± SEMs. *nd*, Not detectable; *ns*, not significant. *$P < .05$ and **$P < .01$.

To study the role of HFD and associated obesity on cutaneous food allergen sensitization, we next assessed production of the canonical type 2 cytokines IL-4, IL-5, and IL-13 in cell-free culture supernatants of allergen-restimulated SLN cells. Although food allergen sensitization on AD-like skin resulted in robust allergen-specific type 2 immune responses in SLNs, no significant changes were observed in mice fed either an SCD or an HFD (Fig 2, *E*).

Together, these data demonstrate that HFD and obesity do not directly affect the development of AD-like skin inflammation and the magnitude of associated cutaneous food allergen sensitization in this experimental setting.

HFD promotes mucosal mast cell accumulation and increases intestinal permeability

Given the absence of an effect on cutaneous inflammation and sensitization (Fig 2) but increased systemic levels of mast cell proteases (Fig 1, *H*), we hypothesized that effector mast cells in the intestine can become dysregulated in the setting of food allergy associated with HFD. To test this, we first examined whether there were differences in mast cell frequencies and numbers in the intestines of SCD- and HFD-fed nonallergic mice (EtOH plus OVA) and found no differences across groups (Fig 3, *A-C*). Strikingly, when experimental food allergy was induced in both SCD- and HFD-fed mice (MC903 plus OVA), there was a significant increase in intestinal mast cell accumulation in HFD-fed mice compared with control animals, whereas no significant changes in eosinophil frequencies and numbers were observed (Fig 3, *A-C*). Visualization and enumeration of chloroacetate esterase–positive mast cells in small intestinal tissue sections corroborated flow cytometric findings with HFD feeding, potentiating food allergy–related intestinal mast cell accumulation (Fig 3, *D* and *E*).

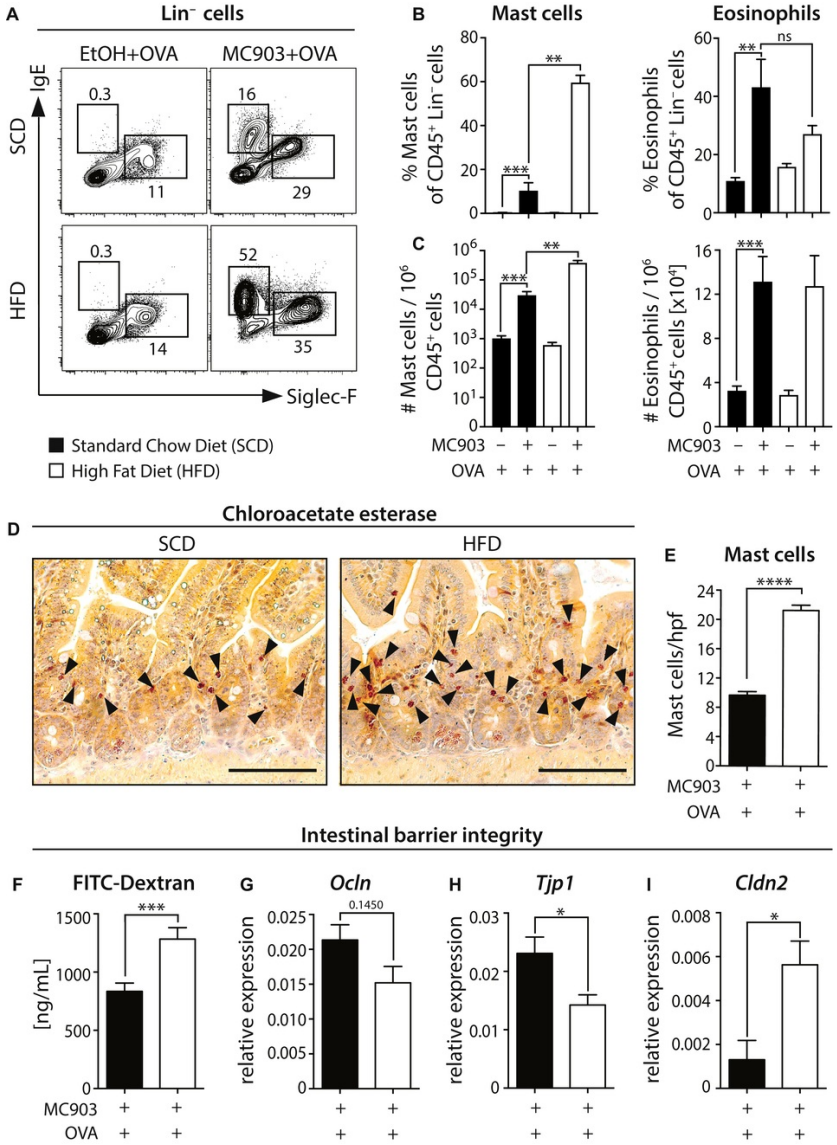

Fig 3 HFD feeding potentiates food allergy–related mucosal mast cell accumulation and increases intestinal permeability. **A,** Representative fluorescence-activated cell sorting plots of mast cells and eosinophils in the small intestinal lamina propria of control mice (EtOH plus OVA) c mice with food allergy (MC903 plus OVA) fed an SCD or HFD. **B,** Quantification of mast cell and eosinophil frequencies in the small intestinal lamina propria. For quantification, mast cells were gated as live $CD45^+$, and Lin^- ($CD3^-CD5^-CD19^-NK1.1^-$) cells coexpressing c-Kit and IgE. Eosinophils were gated as live $CD45^+$, Lin^- ($CD3^-CD5^-CD19^-NK1.1^-$) Siglec-F^+, and side scatter–high cells. **C,** Quantification of total mast cell and eosinophil numbers in the small intestinal lamina propria. **D,** Chloroacetate esterase staining of mast cells from the jejunum. *Scale bar* = 100 μm. **E,** Quantification of chloroacetate esterase–positive mast cells per high-powered field *(hpf)*. **F,** FITC-Dextran concentration in serum of mice with food allergy fed an SCD or HFD. **G-I,** Gene expression levels of the tight junction genes *Ocln*, *Tjp1* and *Cldn2* in the small intestines of SCD- or HFD-fed mice with food allergy. *Solid bars* represent mice fed an SCD, and *open bars* represent HFD-fed mice. Data in Fig 3, *A-E* and *G-I*, are representative of 4 independent experiments (pooled) with 4 to 6 mice per group. Data in Fig 3, *F*, are representative of 2 individual experiments with 5 to 8 mice per group. *Error bars* indicate means ± SEMs. *ns*, Not significant. $*P < .05$, $**P < .01$, $***P < .005$, and $****P < .001$.

We next tested whether enhanced mast cell accumulation and associated release of mast cell–specific proteases might further promote reported HFD-induced intestinal permeabilization. Consistent with previous reports,[14,52,53]

we found that HFD feeding altered intestinal permeability, as assessed by an *in vivo* FITC-Dextran assay, which was further enhanced on induction of food allergy in HFD-fed mice (Fig 3, *F*, and data not shown). Altered intestinal barrier integrity in HFD-fed mice with food allergy further correlated with a reduction in gene expression levels of occludin *(Ocln)* and tight junction protein 1 *(Tjp1)* and a significant increase in expression of claudin-2 *(Cldn2)*, a channel-forming tight junction protein expressed predominantly in leaky epithelia (Fig 3, *G-I*). Together, mice with food allergy fed an HFD presented with dysregulated intestinal mast cell responses and increased intestinal permeabilization that can promote facilitated passage of allergens to perpetuate intestinal allergic inflammation.

HFD-induced adiposity and food allergy are associated with intestinal dysbiosis

In addition to increasing intestinal barrier permeability, HFD feeding and associated obesity have been demonstrated to promote significant changes in the relative abundance and phylogenetic diversity of the gut microbiota.[18,54-56] Consistent with these previous reports, 16S rDNA amplicon sequencing analyses confirmed that high dietary fat intake is associated with pronounced changes in gut microbiota composition (Fig 4, *A*, and see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). We found an overall reduction in α-diversity, with fewer and more dominant OTUs in mice fed an HFD compared with SCD-fed control mice, as assessed based on the total number of OTUs and a Shannon index result that is further compromised in HFD-fed mice with food allergy (Fig 4, *B* and *C*). Using the Welch test to detect per-family and per-genera differences across different treatments, we observed a significant increase in abundance of the families Ruminococcaceae (Clostridiales), Desulfovibrionaceae (Deltaproteobacteria), and Rikenellaceae (Bacteroidetes) and a reduction in abundance of the families Muribaculaceae and Prevotellaceae in mice fed an HFD diet ($P < .05$, difference between proportions > 1, ratio between proportions > 2; see Fig E3, *B*). The observed changes in α-diversity in response to HFD feeding suggest an increase in dominance of specific phylotypes (relative abundances of Verrucomicrobia, Deltaproteobacteria, Ruminococcus and Lachnospiraceae [Clostridia], and other Bacteroidaceae), a pattern that is consistent with previous reports addressing the effect of high dietary fat intake on gut microbiota composition.[27,57]

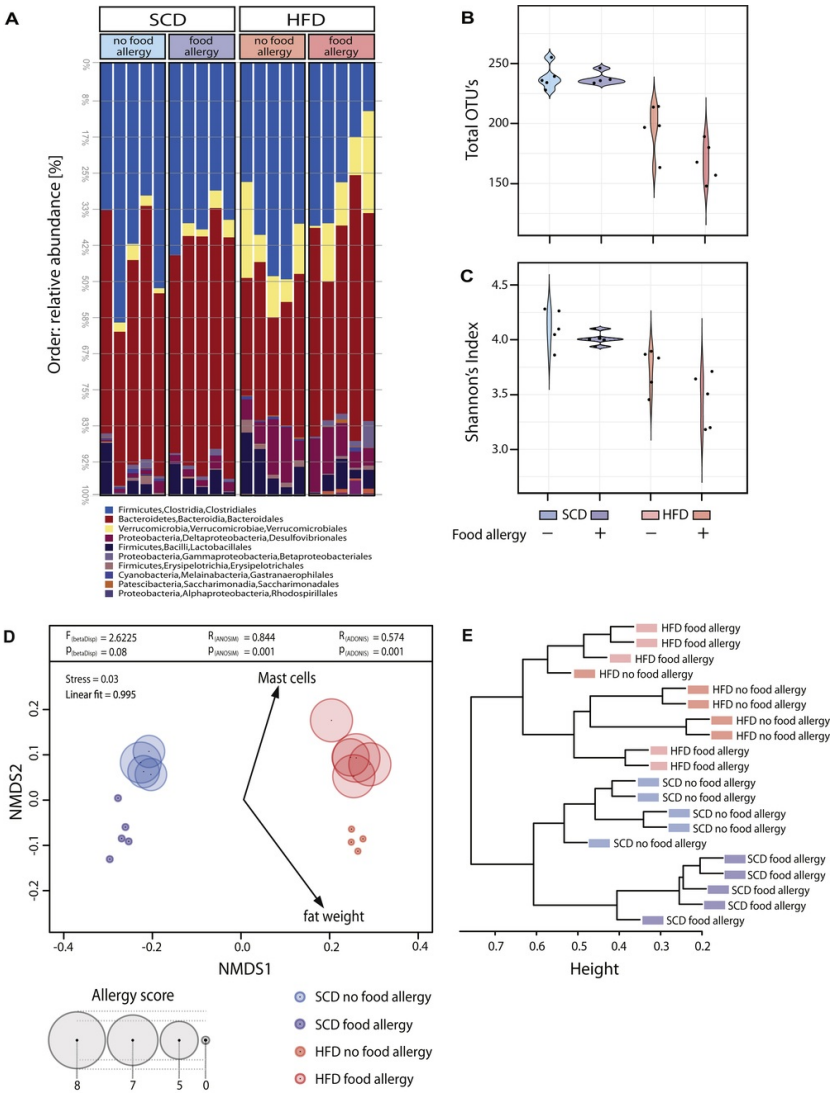

Fig 4 Effects of diet-induced obesity and food allergy on gut microbiota composition. **A,** Bacterial relative abundances represented at the order level. **B,** Microbial richness, as assessed by total OTU numbers. **C,** Shannon index. **D,** NMDS plot of gut microbiota composition, intestinal mast cells, and adipose tissue weight of nonallergic mice and mice with food allergy fed an SCD or HFD. Animals are represented by *dots*, and treatment is color coded. Circle size indicates the magnitude of the allergy score of each animal. The number of intestinal mast cells and weight of epididymal fat fitted variables are represented by *black vectors*. Differences between treatments are supported by ANOSIM and ADONIS, and statistics are depicted in the *upper panel*. **E,** Average clustering dendrogram. All OTUs in Fig 4, *A* and *B*, show significant differences ($P < .05$, differences between proportions > 1, and ratio of proportions < 2).

To assess β-diversity across samples, we next used NMDS analysis as a commonly regarded robust unconstrained ordination method in community ecology. NMDS plots show a significant separation of sample groups according to dietary manipulation and allergic disease state (Fig 4, *D*). We observed that an HFD modulates the bacterial profile and draws this group apart from the microbiota profile of mice fed a control diet. Furthermore, using multivariate analyses, we found that epididymal fat weight, frequency of intestinal mast cells, and clinical allergy scores significantly correlate with microbial clustering patterns with the length of the vectors (mast cells and fat weight) and sizes of circles (allergy score) corresponding to the importance of each of the parameters assessed to the clustering pattern. An average clustering dendrogram of Bray-Curtis dissimilarities further demonstrates the distance and clustering of

individual samples according to both dietary modulation and disease state (Fig 4, *E*).

Together, our multivariate analyses exploring the effect of an HFD on gut microbial ecology and food allergy reveal a specific HFD-specific microbiota signature that significantly correlates with disease markers and clinical signs of experimental food allergy.

HFD-associated microbiome is transmissible and durable

We next performed microbiota transplantation experiments to test a potential disease-promoting role of a signature HFD microbiota for the pathogenesis of food allergy. To do so, 4-week-old wild-type C57BL6/J GF mice were horizontally colonized by cohousing GF mice with microbiota donors fed either a control diet (SCD) or an HFD (1 donor plus 4 GF mice per treatment group per experiment, 2 independent experiments). Recipients were colonized with the respective donor microbiotas for 2 months and fed a control diet (SCD) throughout the experiment (Fig 5, *A*). We found that the microbiota phenotype of HFD-fed mice (reduced α-diversity and increased β-diversity) is transmissible to microbiota-replete GF recipients (Fig 5, *B-D*, and see Fig E4 in this article's Online Repository at www.jacionline.org). Importantly, an HFD microbiota signature was conserved over time in GF reconstituted mice fed an SCD, suggesting that there are postdieting differences in the microbiota composition or function that cannot be readily reverted, even if mice are returned to their original diet. NMDS plots and an average clustering dendrogram of β-diversity show that the microbiota of reconstituted GF mice clustered in accordance with the microbiota input source, with the largest Bray-Curtis dissimilarities observed between diet treatment of the source communities ($P < .001$; Fig 5, *E* and *F*).

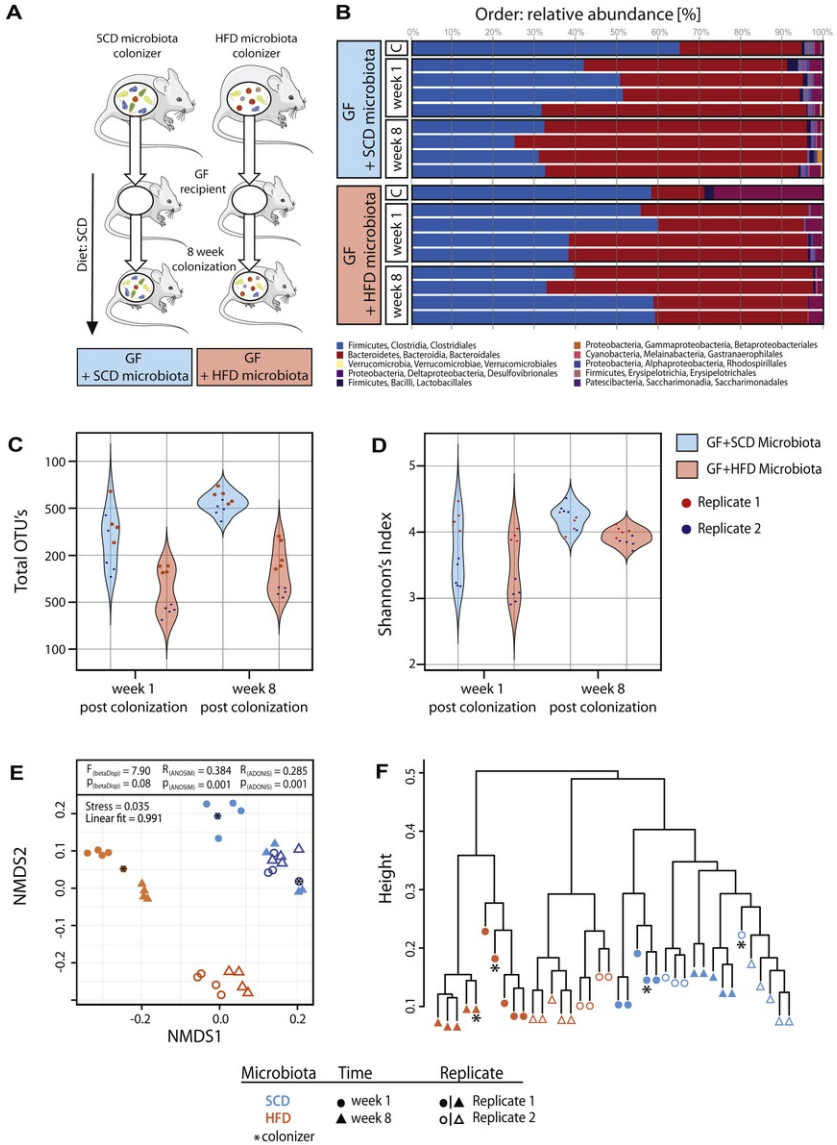

Fig 5 Reconstitution of GF mice with a donor lean or HFD-associated microbiome. **A,** Schematic of microbiota repletion studies. **B,** Bacterial relative abundances represented at the order level. **C,** Microbial richness as assessed based on total OTU numbers. **D,** Shannon index. **E,** NMDS plot of gut microbiota composition of microbiota-replete GF mice colonized with an SCD- or HFD-associated microbiome at week 1 and week 8 after colonization. *Asterisks* indicate colonizer microbiomes. Differences between treatments are supported by ANOSIM and ADONIS, and statistics are depicted in the *upper panel*. **F,** Average clustering dendrogram. Graphs in Fig 5, *C-F*, show combined data of 2 individual experiments.

Together, our microbiota transplantation experiments suggest that an HFD-associated microbiome is transmissible to GF mice and clusters with the donor microbiota through time. Such inherited dietary-induced changes in the gut microbial community structure can have significant functional properties on host physiology and immunity independently of diet.

HFD-associated microbiota confers enhanced susceptibility to food allergy independently of obesity

Although numerous studies have linked specific disease states with changes in the gut microbiota structure, there are limited data demonstrating causality. To explore whether transmission of the HFD-associated microbiota phenotype has functional implications on food allergy pathogenesis, 4-week-old GF mice were colonized with a signature control- or HFD-associated microbiota from lean and obese mice, respectively, for 2 months before induction of food allergy. In contrast to previous studies, obesity did not represent a transmissible trait in GF mice reconstituted with a signature HFD microbiota over the course of 2 months. Irrespective of the donor microbiota input, microbiota-replete GF mice exhibited comparable weight gain and epididymal fat mass accumulation over time (Fig 6, *A*, and see Fig E5 in this article's Online Repository at www.jacionline.org). Furthermore, histologic analyses of adipocyte size in epididymal adipose tissue did not reveal differences between SCD- or HFD microbiome-associated food allergic mice. (Fig 6, *B* and *C*). Intriguingly, we find that increased susceptibility to food allergy in mice fed an HFD was transmissible to GF mice when reconstituted with a donor HFD microbiota. GF mice reconstituted with a signature HFD microbiota exhibited increased food-induced allergic responses compared with control mice (GF mice colonized with a signature lean microbiota), as characterized by increased serum IgE levels (Fig 6, *D* and *E*), increased MMCP-1 levels (Fig 6, *F*), type 2 intestinal cytokine responses (Fig 6, *G*), and accumulation of effector mast cells to the small intestinal lamina propria, as assessed by using flow cytometry (Fig 6, *H*) and chloroacetate esterase tissue staining (Fig 6, *I* and *J*).

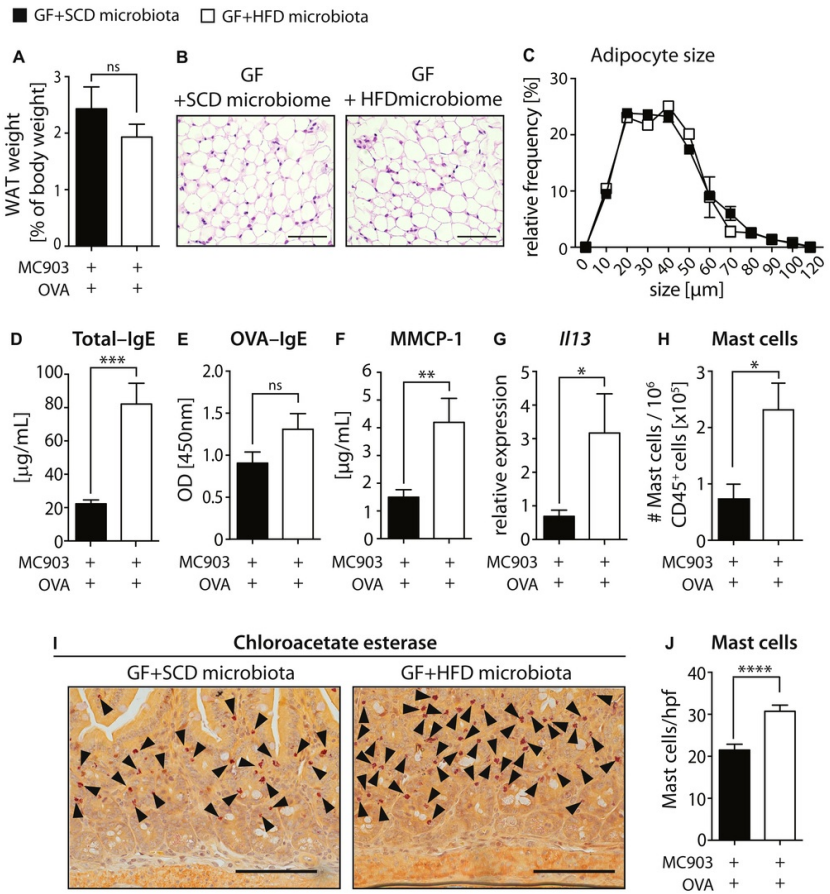

Fig 6 An HFD-associated microbiome confers enhanced susceptibility to food allergy. **A,** WAT weight of percentage body weight of GF mice reconstituted with an SCD- or HFD-associated microbiome. **B,** H&E staining of WAT. *Scale bar* = 100 µm. **C,** Distribution of adipocyte size of WAT, as determined by using histomorphometric analyses. **D** and **E,** Total (Fig 6, *D*) and OVA-specific (Fig 6, *E*) serum IgE levels in ex-GF mice with food allergy reconstituted with an SCD- or HFD-associated microbiome. **F,** Serum MMCP-1 levels. **G,** Relative gene expression levels of *Il13* in the small intestine. **H,** Mast cell numbers in the small intestinal lamina propria, as assessed by using flow cytometry. **I,** Chloroacetate esterase staining of mast cells from the jejunum. *Scale bar* = 100 µm. **J,** Quantification of chloroacetate esterase–positive mast cells per high-powered field (hpf). Data are representative of 2 independent experiments with 4 to 5 mice per group. *Solid bars* represent GF mice reconstituted with an SCD-associated microbiome, and *open bars* represent HFD microbiome–reconstituted mice. *Error bars* indicate means ± SEMs. *ns*, Not significant. **P* < .05, ***P* < .01, ****P* < .005, and *****P* < .001.

Together, our studies demonstrate that a microbiota signature associated with high dietary fat intake has disease-promoting properties by conferring increased susceptibility to experimental food allergy independently of an obese phenotype.

Discussion

The present study provides several conceptual advances in our understanding of a potential correlation between high dietary fat intake, obesity, and food allergy. First, we report that HFD feeding promotes intestinal mast cell dysregulation in the setting of food allergy. Second, we demonstrate that high dietary fat intake decreases microbial diversity, a trait that is both transmissible and durable. Third and finally, HFD-associated dysbiosis confers enhanced susceptibility to food allergy independently of obesity. Collectively, our findings indicate that high dietary fat intake enhances susceptibility to food allergy and that this effect is mediated by durable postdieting changes in the gut microbiome.

A Western diet rich in unsaturated fats and refined sugars has been hypothesized as a contributing factor to the surge in allergies in pediatric patients.[58,59] Furthermore, several clinical studies have demonstrated a positive correlation of body mass index with IgE levels and a greater incidence of atopy in obese children.[60,61] However, how diet and obesity predispose to allergic disorders remains ill-defined.

Here we demonstrate that high dietary fat intake enhances food allergy. Although HFD feeding had no effect on intestinal mast cells at steady state, induction of allergic inflammation in this setting resulted in robust accumulation of mucosal mast cells, which has previously been shown to correlate with disease severity.[37] However, the mechanisms by which mast cells expand in the setting of HFD feeding and food allergy remain poorly defined. Previous studies suggested white adipose tissue (WAT) as a dedicated reservoir of mast cell progenitors (MCps) that have the capacity to migrate to distant organs, such as the intestine.[62] Although HFD feeding in our experimental settings did not alter MCps in the intestine (data not shown), we found a significant increase in numbers of MCps in WAT of HFD-fed mice compared with control mice (see Fig E6 in this article's Online Repository at www.jacionline.org). It is likely that in the context of HFD feeding and subsequent induction of experimental food allergy, WAT-derived MCps migrate to sites of allergic inflammation. However, additional studies are required to support an MCp WAT-gut axis in the context of HFD feeding.

Our findings demonstrating that HFD feeding induces gut dysbiosis, which in term promotes food allergy, suggest that commensal alterations result in modulation of mast cell proliferation, recruitment, or both. It is possible that this occurs either directly or indirectly through effects on other cells caused by alterations in metabolites, such as the availability of short-chain fatty acids. Indeed, recently, it has been shown that short-chain fatty acids promote intestinal regulatory T cells to inhibit mast cell expansion and degranulation.[54]

Yet another mechanism that might explain exaggerated mast cell accumulation in mice with food allergy fed an HFD could involve IgE binding on mast cells. Importantly, reduced gut microbial diversity, as observed in HFD-fed mice, has been associated with hyper-IgE responses.[63,64] Despite the induction of allergen-specific IgE responses in control- and HFD-fed mice with food allergy being comparable, total serum IgE levels were increased in obese mice with food allergy but did not reach statistical significance. Nevertheless, increased total IgE responses in mice with food allergy fed an HFD can stabilize mast cell function and survival, thus explaining exaggerated mucosal mast cell accumulation in HFD-fed mice with food allergy.[65] However, future studies will be required to determine how HFD-associated dysbiosis promotes mast cell accumulation in the intestines of mice with food allergy.

Consistent with previous reports, HFD feeding in our experimental settings was associated with a significant decrease in α-diversity, with increased relative abundance of specific phylotypes, including Verrucomicrobia, Deltaproteobacteria, Ruminococcus, Lachnospiraceae (Clostridia), and other Bacteroidaceae.[27,57] Although HFD feeding has been demonstrated to promote significant changes to the gut microbial community structure within a few days, such dietary-induced changes to the gut microbiota can be partially reversed by subsequent low-fat, high-fiber dietary manipulations.[18] However, long-term dietary interventions can irreversibly disrupt a core gut microbial community structure with long-lasting consequences for the host. In our experimental settings we found that even after the introduction of a control diet, once established in recipient mice, microbiome signatures persisted and were durable. Consistent with our experiments, studies by Thaiss et al[66] demonstrated that HFD-induced changes in the gut microbial community structure persisted for months, even if cycles of HFD feeding were interspersed with periods of low-fat diets. In light of these findings, postdieting microbiome alterations and associated dietary-induced losses in key microbial functions might be difficult to reverse, with potentially profound consequences for host immunity and metabolism.[67,68]

In addition to identifying that HFD promotes intestinal dysbiosis, we found that transfer of an HFD-specific microbiota also conferred enhanced susceptibility of food allergy in microbiota replete GF mice. Strikingly, this process occurred independently of any signs of adiposity in recipient mice. These findings strongly suggest that dietary alteration alone is sufficient to drive microbiota-dependent effects on the intestinal immune system independently of host metabolic derangement. It is important to note that studies by Turnbaugh et al[17,18] reported that transplantation of a Western diet–associated gut microbiome into GF mice increased total body fat and resulted in signs of adiposity. In our experimental settings transfer of HFD-associated microbiota did not reveal an obese phenotype in GF mice. Given the complexity of microbiome studies, differences in founder colonies and colonization protocols (cohousing vs cecal microbiota transplants) are likely to explain these discrepancies. Notwithstanding this, future studies will be required to define which microbial populations specifically promote obesity, whereas others promote allergic susceptibility.

Previous work and our studies herein suggest that HFD feeding and induction of experimental food allergy promote intestinal permeabilization, with potential widespread consequences for host metabolism and immunity.[3] Although our findings demonstrate that HFD feeding induces a microbiota signature that promotes food allergy susceptibility, no significant alterations in expression levels for barrier-related genes were observed in GF-replete mice with food allergy colonized with an HFD-associated microbiome compared with control mice (see Fig E7 in this article's Online Repository at www.jacionline.org). It is likely that intestinal barrier integrity in response to a HFD-associated microbiome is transiently regulated, and therefore no significant alterations at the assessed time points are observed. Alternatively, increased allergy susceptibility in HFD microbiota–replete GF mice might not be directly related to alterations in intestinal barrier integrity. Additional studies are required to assess a correlation between food allergy susceptibility and intestinal barrier function in response to an HFD-associated microbiome.

Although the microbial community structure in recipient GF mice differed between experiments, likely because of individual donor microbiomes, increased susceptibility to experimental food allergy was conserved in HFD microbiota–replete GF mice across experiments. Although we find differences in total OTU numbers between experimental replicates as a likely result of differences in survival of reinoculated bacteria, a uniform pattern toward recovery of the community structure is observed across experiments. After comparing results from individual microbiota transplantation experiments, we consistently identified members of the family Lachnospiraceae and the Rikenellaceae RC9 groups to be overrepresented in microbiota replete GF mice colonized with an HFD-associated gut microbiome, whereas members of the family Muribaculaceae and the Prevotellaceae group UCG-001 were underrepresented compared with control mice. Although it is tempting to propose individual roles for OTUs, it is likely that the differential effect over the immune system varies between strains and that the same response can be caused by different strains from different taxonomic backgrounds. Alternatively, a decrease in overall microbial diversity as a result of prolonged high-fat dieting rather than overrepresentation or underrepresentation of specific bacterial strains might be sufficient to favor allergic inflammation. Consistent with this hypothesis, GF mice or mice harboring a reduced gut microbial community structure (eg, ASF mice, antibiotic treated mice) are more susceptible to allergic inflammation. Importantly, restoration of gut microbial complexity has proved sufficient to limit hyper-IgE responses and allergic inflammation.[15,63,64] However, it remains to be tested whether restoring an HFD-mediated decrease in microbial diversity with fecal microbiota transplants from control diet–fed donors or by reconstitution with selected microbial strains might alter food allergy susceptibility. Despite the profound effect of diet on gut bacterial community structure, future work should also track changes in interkingdom relationships on dietary manipulations because disruption of the mycobiome has been associated with increased susceptibility to allergic inflammation.[69,70]

In conclusion, our study identifies HFD-induced microbial alterations as a risk factor for experimental food allergy and uncouples a pathogenic role of an HFD-associated microbiome from obesity. Restoring microbial diversity in children on a Western diet might represent a promising approach to limit allergy susceptibility in genetically predisposed subjects.

Key messages

- Diet-induced obesity increases susceptibility to experimental food allergy.
- Overindulgence of dietary fat decreases gut microbial diversity, a trait that is both transmissible and durable.
- An HFD-associated microbiome confers enhanced susceptibility to food allergy independently of obesity.

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Appendix

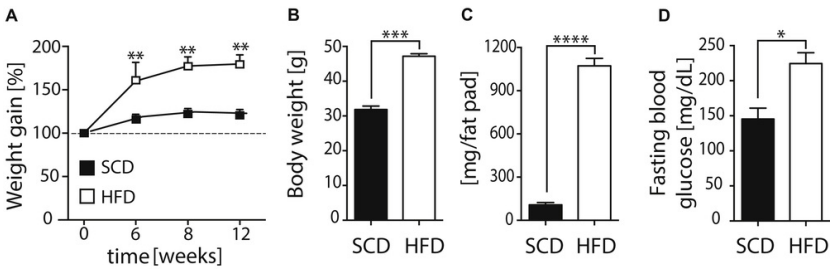


Fig E1 Assessment of diet-induced obesity. **A,** Percentage weight gain in response to HFD feeding over time. **B,** Body weight. **C,** Weight of epididymal fat pads. **D,** Fasting blood glucose levels. Data are representative of 4 independent experiments with 4 to 6 mice per group. *Error bars* indicate means ± SEMs. **P* < .05, ***P* < .01, ****P* < .005, and *****P* < .001.

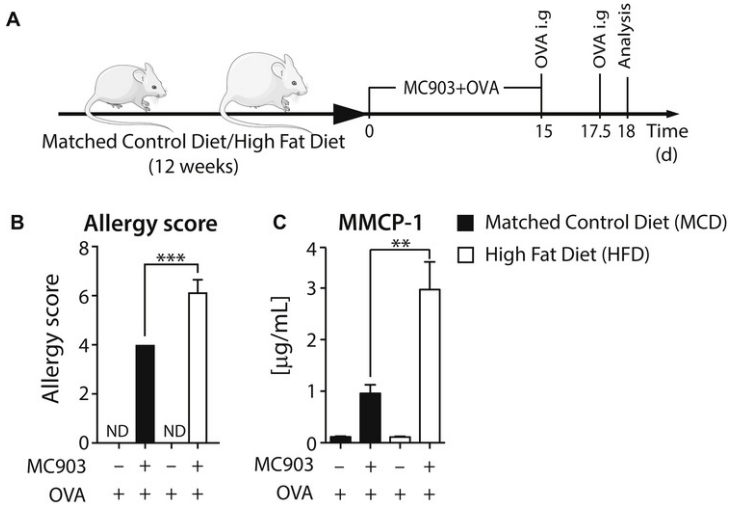


Fig E2 Food allergy induction in mice fed an HFD or a matched control diet. **A,** Experimental protocol. *i.g.,* Intragastric. **B,** Clinical allergy score of control mice and mice with food allergy fed a matched control diet or an HFD. **C,** Serum MMCP-1 levels. Data are representative of 2 independent experiments with 4 to 5 mice per group. *Solid bars* represent mice fed a matched control diet, and *open bars* represent HFD-fed mice. *Error bars* indicate means ± SEMs. *ND,* No disease. $^{**}P < .01$ and $^{***}P < .005$.

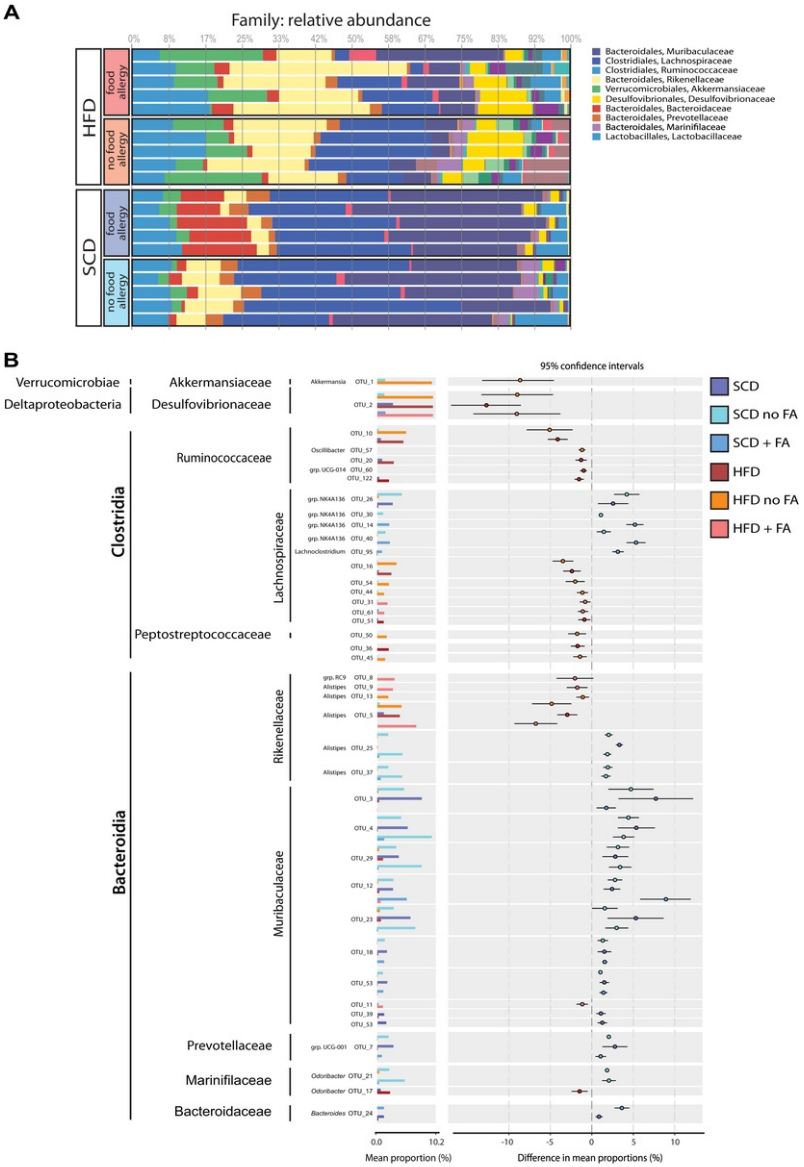


Fig E3 Changes in gut microbial community structure in response to ~~an~~ HFD. **A,** Bacterial relative abundances represented at the family level. **B,** OTUs that show significant differences in abundance across treatments, according to the Welch 2-sided test. OTUs shown all have *P* values of less than .05 (corrected with the Storey false discovery rate) and large effect sizes (differences between proportions > 1 and ratios between proportions > 2). *FA*, ~~■■■~~food allergy.

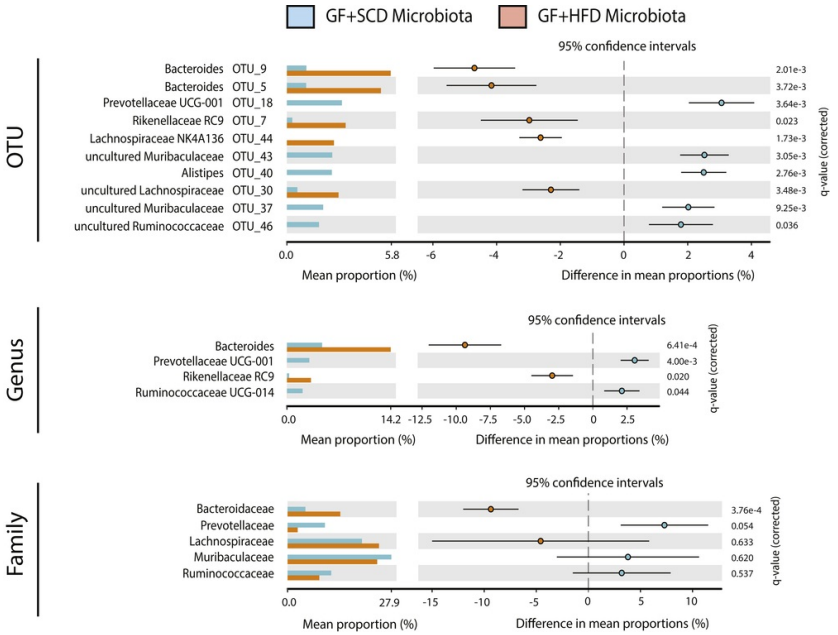


Fig E4 Changes in gut microbial community structure at the OTU, genus, and family levels in microbiota-replete GF mice, according to the Welch 2-sided test. Only differences with large effect sizes (differences between proportions > 1 and ratios between proportions > 2) are shown.

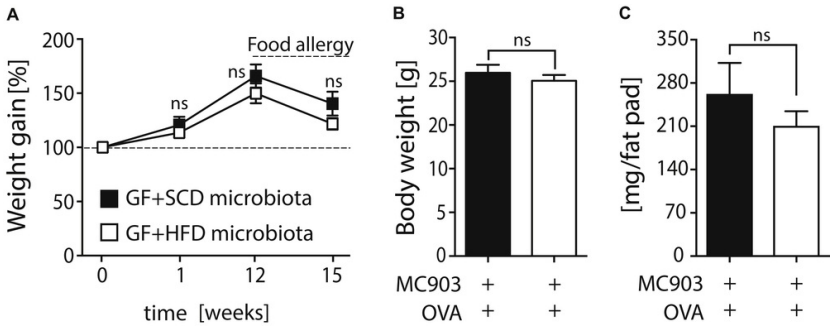


Fig E5 An HFD-associated microbiome does not promote weight gain. **A,** Percentage body weight gain of GF mice reconstituted with an SCD- or HFD-associated microbiome. **B,** Body weight. **C,** Weight of epididymal fat pads. Data are representative of 2 independent experiments with 4 to 5 mice per group. *Solid bars* represent GF mice reconstituted with an SCD-associated microbiome, and *open bars* represent HFD microbiome–reconstituted mice. *Error bars* indicate means ± SEMs. *ns,* Not significant.

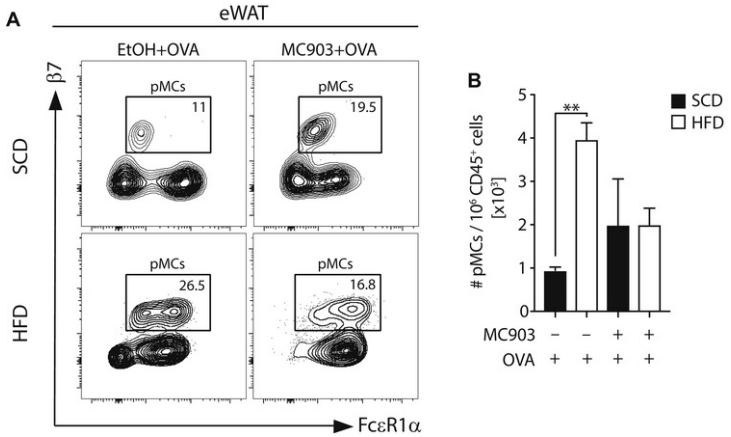


Fig E6 Assessment of MCps in WAT of SCD or HFD fed mice. **A,** Representative flow cytometric plots of MCps in WAT of control mice or mice with food allergy fed an SCD or HFD. *eWAT,* Epididymal WAT. **B,** WAT MCp numbers. Data are representative of 2 individual experiment with 3 to 5 mice per group. *Error bars* indicate means ± SEMs. $^{**}P \leq .01$.

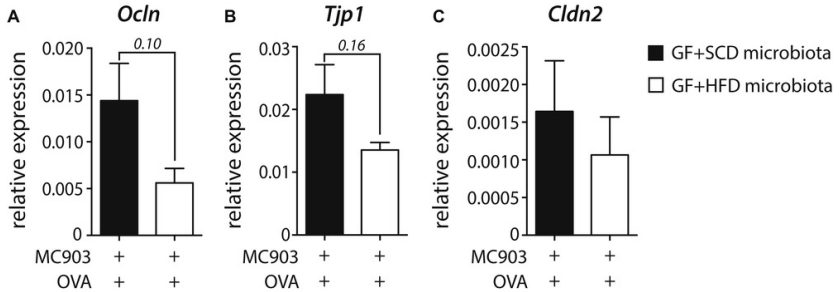


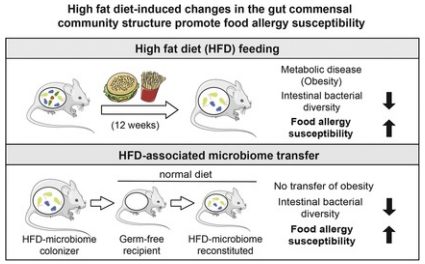
Fig E7 Gene expression levels for intestinal barrier–associated genes in microbiome-replete mice with food allergy. Gene expression levels of *Ocln* **(A)**, *Tjp1* **(B)**, and *Cldn2* **(C)** in the small intestines of microbiota-replete GF mice with food allergy. Data are representative of 2 independent experiments with 4 mice per group. *Solid bars* represent GF mice with food allergy reconstituted with an SCD-associated microbiome, and *open bars* represent HFD microbiome–reconstituted mice with food allergy. *Error bars* indicate means ± SEMs.

Table E1 Description of clinical allergy score

Score	Description
Scratching	
0	No symptoms
1	Mild scratching; rubbing of nose, head, or feet (<5 episodes)
2	Intermediate scratching; rubbing of nose, head, or feet (>5 to <10 episodes)
3	Severe scratching (>10 episodes)
Behavior	
0	Normal
1	Hyperactivity
2	Aggressive behavior; pain loud after prodding

Physical appearance	
0	Normal activity
2	Significantly reduced mobility; piloerection
3	Immobility after prodding, tremors, and/or significant respiratory distress
Stool consistency	
0	Normal
2	Loose stool
4	Diarrhea

Graphical abstract



Queries and Answers

Query: If there are any drug dosages in your article, please verify them and indicate that you have done so by initialing this query
Answer: does not apply

Query: Please verify that WAT is spelled out correctly here as “white adipose tissue.”
Answer: correct

Query: Please clarify the “Kit protocol.” Should this have a citation or manufacturer’s information?
Answer: QIAmp Fast DNA Stool Kit, Qiagen

Query: Please verify that data on quality filtering of raw reads is presented clearly and completely as shown (ie, please spell out “-p,” “-m,” etc). Also, if applicable, please provide a citation for Zhang et al, citing it in numeric order and renumbering all subsequent citations throughout.
Answer: Raw reads were quality-filtered with FastX-Tookit (a minimum quality score of 33 kept across 75% of the bases), merged with PEAR (with a maximum possible length of merged reads of 360, minimum possible length of merged reads of 100, score quality threshold for trimming of 26, minimum overlap size of 50, and a base PHRED quality score of 33

Query: Please spell out ADONIS and ANOSIM throughout, if applicable.
Answer: These are standard statistical tests!

Query: Please note that *P* values indicated by asterisks have been moved to figure legends per journal style. Please check carefully.
Answer: ok

Query: Please provide volume number for reference 46.

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Query: Please provide volume and page numbers for reference 56. It does not appear in PubMed.

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Query: Please spell out FA in the legend fir Fig E3.

Answer: FA: food allergy

Query: Have we correctly interpreted the following funding source(s) and country names you cited in your article: Human Frontier Science Program, France; Fondation Acteria, Switzerland; European Research Council, European Union?

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Answer: Yes